Energy Conserving Hydrogenases Drive Syntrophic Growth of *Desulfovibrio vulgaris* and *Methanococcus maripaludis*

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ESPP Environmental Stress Pathway Project

DOE GENOMICS:GTL SYSTEMS BIOLOGY FOR ENERGY AND ENVIRONMENT — OFFICE OF SCIENCE, U.S. DEPARTMENT OF ENERGY

VIMSS Virtual Institute for Microbial Stress and Survival — http://vimss.lbl.gov

ABSTRACT

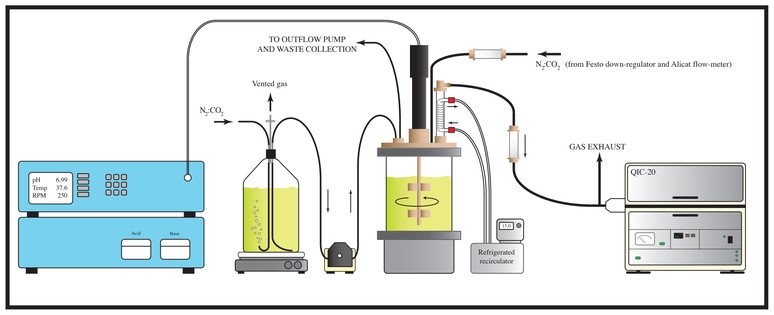
In the absence of an electron acceptor, many *Desulfovibrio* species grow on non-fermentable substrates via syntrophic association with hydrogen consuming methanogens. Building upon the ongoing Virtual Institute for Microbial Stress and Survival (VIMSS) investigation into the response of *Desulfovibrio vulgaris* Hildenborough to environmental stressors found in contaminated DOE sites, the Environmental Stress Pathway Project's (ESPP) Applied Environmental Core (AEC) developed and maintained a stable syntrophic consortium. *Desulfovibrio vulgaris* Hildenborough and *Methanococcus maripaludis* LL were continuously grown in a chemostat on minimal media amended with lactate but lacking electron acceptor. Replicated whole genome transcriptional analyses by the ESPP Functional Genomics Core (FGC) and the Computational Core (CC) identified 169 and 254 genes that were significantly up- or down-regulated, respectively, relative to a sulfate-limited monoculture growing at the same generation time. The majority of up-regulated genes were associated with energy production/conservation, signal transduction mechanisms, and amino acid transport/metabolism. A number of the down-regulated genes were associated with signal transduction mechanisms, inorganic ion transport/metabolism and aminio acid transport and metabolism. Among those genes most highly up-regulated were a suite of hydrogenases including the putative carbon-monoxide induced hydrogenase (Coo, DVU2286-93). Coo is a multi-subunit membrane-bound complex with high similarity to an energy conserving protein in *Rhodospirillum rubrum*. In order to further elucidate the possible role energy conserving hydrogenases play in syntrophic growth, we examined transposon mutants generated by theFGC of both the Coo hydrogenase (*cooL*) and a structurally related homolog, Ech (energy conserving hydrogenase, *echA*, DVU0429-34). Both mutants grew to the same cell density on lactate/sulfate, although the *cooL* mutant grew slower. When grown in coculture with *M. maripaludis* without any sulfate, the *cooL* mutant grew significantly slower and to approximately 25% yield, while the *echA* mutant showed a less pronouced difference in growth rate and yield (approximately 80%). Together these data suggest an important role for the Coo hydrogenase in energy conservation of *D. vulgaris* Hildenborough during syntrophic growth, possibly through proton translocation, although the exact physiological mechanism remains to be elucidated. Continued collaborative work by the VIMSS three ESPP core groups should provide a more complete mechanistic understanding of sulfate-reduction and syntrophic coordination between microbes.

TRANSCRIPTIONAL RESPONSE

Gene	TIGR annotation	Log R	|ZI| score	RNA/gDNA expression ratio in coculture
DVU2285	lactate permease	1.37	1.92	0.44
DVU3025	pyruvate-ferredoxin oxidoreductase (*por*)	1.96	2.51	1.82
DVU1179	aldehyde-ferredoxin oxidoreductase (*aor*)	1.93	1.91	1.64
DVU2287	Coo hydrogenase (*cooL*)	1.67	2.56	1.88
DVU2289	Coo hydrogenase (*cooU*)	1.73	2.77	1.56
DVU2402	heterodisulfide reductase (*hdrA*)	1.42	2.03	0.29
DVU2405	alcohol dehydrogenase	2.94	3.51	3.39
DVU1769	periplasmic [Fe] hydrogenase (*hydA*)	2.33	1.86	0.37
DVU1922	periplasmic [NiFe] hydrogenase (*hynA*)	0.96	1.09	0.23
DVU0536	high-molecular weight cytochrome (*hmcA*)	2.22	2.85	0.54
DVU0531	high-molecular weight cytochrome (*hmcF*)	1.93	2.76	0.26
DVU1295	sulfate adenylyltransferase (*sat*)	0.75	1.17	4.48
DVU0847	adenylyl-sulfate reductase (*apsA*)	1.21	1.20	4.84
DVU0402	dissimilatory sulfite reductase (*dsrA*)	0.94	1.56	3.59
DVU1636	pyrophosphatase (*ppaC*)	1.38	1.82	3.16
DVU0173	thiosulfate reductase (*phsA*)	2.08	3.41	0.48
DVU0053	sulfate permease, putative	-0.84	1.43	0.07
DVU3107	cytochrome c family protein	-1.82	2.32	0.08
DVU2680	flavodoxin	-5.19	6.67	0.04
DVU0694	molybdopterin oxidoreductase	0.98	1.73	0.56
DVU0599	carbon starvation protein A, putative	2.11	2.77	0.82
DVU2652	hypothetical protein	3.07	4.67	2.12
DVU2655	D-alanyl-D-alanine carboxypeptidase	0.75	1.17	0.95

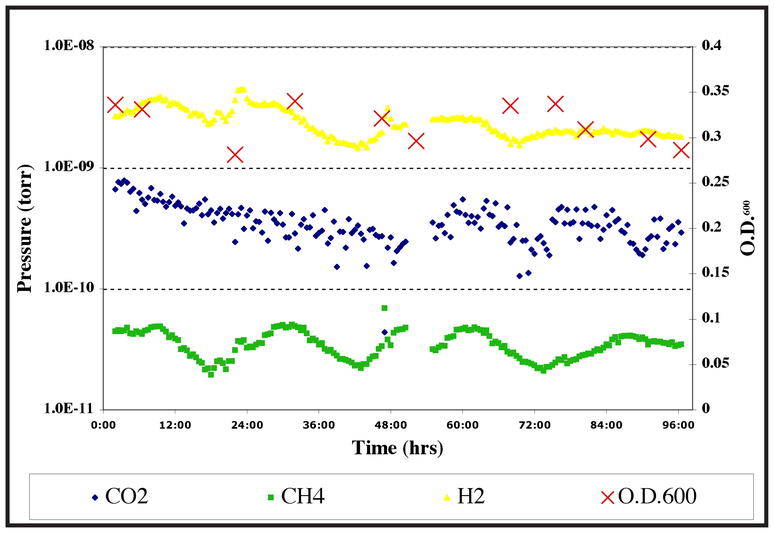
Gene	TIGR annotation	Log R	|ZI| score	RNA/gDNA expression ratio in coculture
DVU2297	glycine/betaine/L-proline ABC transporter	-2.48	3.69	0.45
DVU2298	glycine/betaine/L-proline ABC transporter	-1.99	2.84	0.27
DVU2299	glycine/betaine/L-proline ABC transporter	-2.22	2.90	0.20
DVU2300	hypothetical protein	-1.05	1.20	1.73
DVU2571	ferrous iron transport protein (*feoB*)	-4.71	6.36	0.15
DVU2572	ferrous iron transport protein (*feoA*)	-4.60	7.29	.016
DVU2383	tonB dependent receptor domain protein	-4.37	6.14	0.03
DVU2384	ABC transporter	-2.44	2.5	0.14
DVU2386	ABC trasnporter	-1.05	1.46	0.05
DVU2390	tonB domain protein	-2.34	3.23	0.09
DVU3329	hypothetical protein	-3.00	5.08	0.03
DVU3330	conserved hypothetical proteins	-4.66	6.70	0.07
DVU3331	hypothetical protein	-3.89	5.51	0.05
DVU3332	heavy metal translocating P-type ATPase	-2.15	3.38	0.05
DVU3333	hypothetical protein	-3.11	5.06	0.04
DVU1592	methyl-accepting chemotaxis protein	-1.47	2.51	0.09
DVU1593	chemotaxis protein (*cheY-4*)	-1.61	2.62	0.12
DVU1594	chemotaxis protein (*cheA-3*)	-1.63	2.63	0.10
DVU0806	chemotaxis protein CheYI, putative	-2.59	3.01	0.74

CHEMOSTAT CONFIGURATION



Chemostats are run using a 24 hr retention time at 37 ºC and a stirring speed of 250 rpm. The headspace of the chemostat is flushed with a mixture of N_2 :CO_2 (90:10) at a rate of 0.20 - 0.50 ml/min. The headspace gas composition is sampled in 15 min. intervals using a Hiden QIC-20 mass spectrometer.

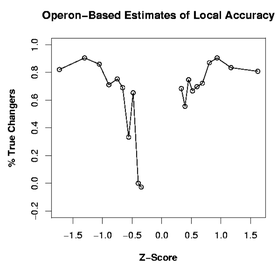
BIOMASS & HEADSPACE GAS MEASUREMENTS

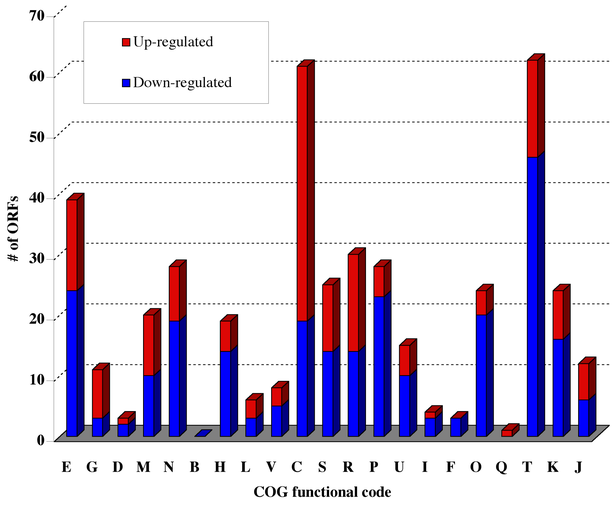


Steady-state was assumed when O.D.$_{600}$ measurements varied by less than 10% of initial value for 3 rentention times. *D. vulgaris*:*M. maripaludis* cell ratio was ~4:1 throughout steady-state as determined by DAPI-stained cell counts.

TRANSCRIPTIONAL ANALYSIS

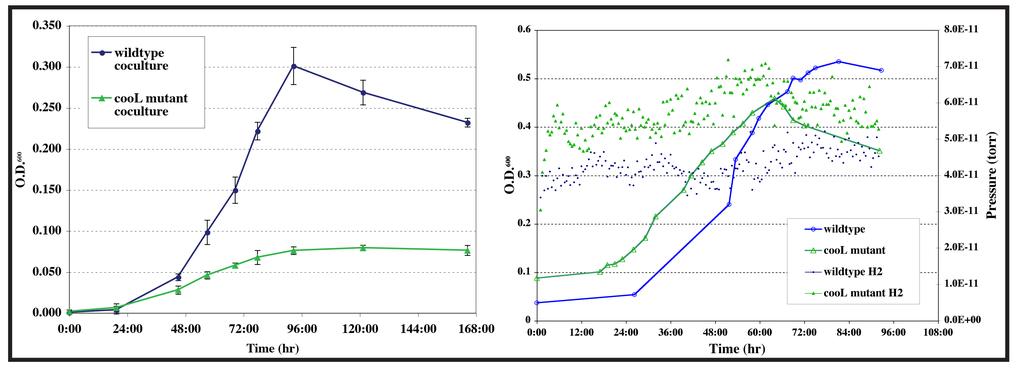
Triplicate biological replicates of cocultures and sulfate-limited *D. vulgaris* monocultures were analzyed by the ESPP FGC using custom-designed whole-genome microarrays. Microarray slides were designed with duplicate spots of each open-reading frame (ORF) for both organisms. At least three slides were used for each biological replicate. The ESPP CC calculated RNA/DNA expression ratos for each ORF and the $\log_2$ ratio comparing the coculture versus sulfate-limited monoculture growth conditions was determined. Z-scores for each ORF were calculated to determine statistical significance. Operon-based estimates of local accuracy indicate an absolute Z-score of 1.0 accurately predicts expression changes between the two conditions. Using this value, 169 ORFs displayed significant up-regulation. 254 ORFs were statistically down-regulated. Genes were assigned clusters of orthologous group (COG) functional codes based on previous genome annotations.



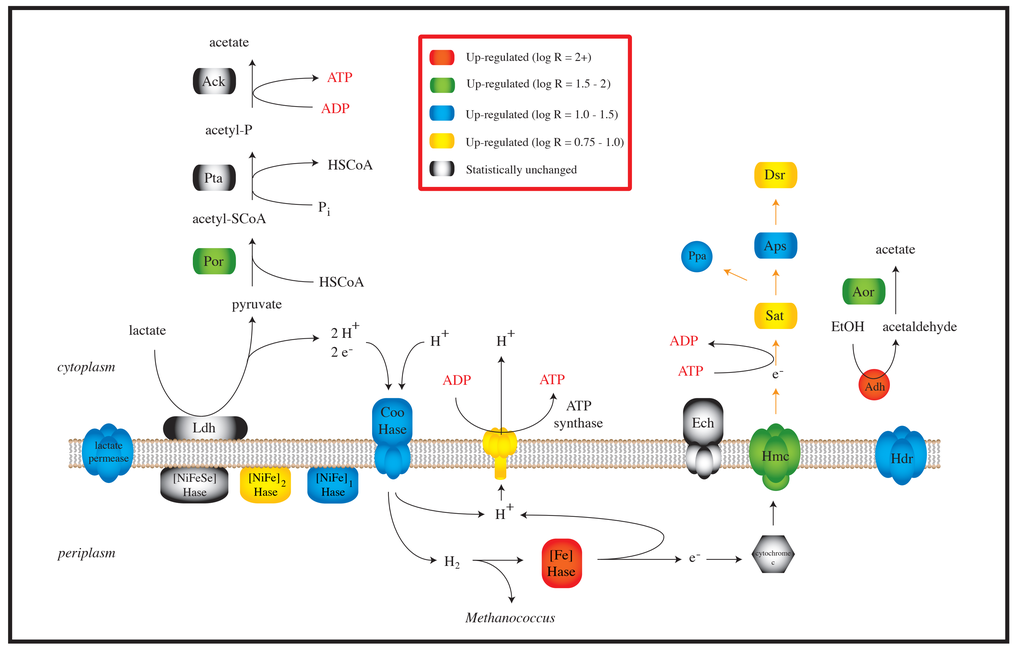


Cluster of orthologous groups up- and down-regulated during syntrophic growth. Categories are amino acid transport (E), carbohydrate transport and metabolism (G), cell division and chromosome partitioning (D), cell envelope biogenesis (M), cell motility and secretion (N), chromatin structure and dynamics (B), coenzyme metabolism (H), DNA replication, recombination and repair (L), defense mechanisms (V), energy production and conservation (C), function unknown (S), general function prediction only (R), inorganic ion transport and metabolism (P), intracellular trafficking and secretion (U), lipid metabolism (I), nucleotide transport and metabolism (F), post-translational modification, protein turnover, chaperones (O), secondary metabolites biosynthesis, transport and catabolism (Q), signal transduction mechanisms (T), transcription (K) and translation, ribosomal structures and biogenesis (J)

PHENOTYPIC DIFFERENCES IN *cooL* MUTANT



ENERGETIC OVERVIEW



ACKNOWLEDGMENT

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